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PATENT TRADEMARK OFFICE

Docket No: 9373/1H812US2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Zhen-Gang WANG et al.

Serial No.: 09/863,765 Art Unit: 1642

Confirmation No.: 9136

Filed: 05/23/2001 Examiner: To be assigned

For: GENE RECOMBINATION AND HYBRID PROTEIN DEVELOPMENT

PRELIMINARY AMENDMENT UNDER 37 C.F.R. § 1.111

Hon. Commissioner of Patents and Trademarks
Washington, DC 20231

Sir:

In accordance with Rule 111 of the Rules of Practice, please enter the following amendment and consider the accompanying remarks before examining the above-captioned patent application. The amendments are made pursuant to the requirements of Rule 121 of the Rules of Practice. Accordingly, Applicants are submitting herewith at Exhibit Tab 1, a marked up copy of each amended paragraph

in the specification showing all changes relative to the specification as originally filed. Applicants also submit herewith a Submission of Substitute Drawings transmittal, including Exhibits A-B with substitute **FIGS. 7, 8, 9-10, 12, 19 and 23** for entry in this application.

It is believed that no fees are required for this amendment. However, should the U.S. Patent and Trademark Office determine that any additional fees are required or that a refund is owed for this application, the Commissioner is hereby authorized and requested to charge the required fee(s) and/or credit the refund(s) owed to Deposit Account No. 04-0100.

Please amend the application as follows:

IN THE DRAWINGS:

Please delete **FIGS. 7, 8, 9-10, 12, 19 and 23** as originally filed for this application and enter, in their place, substitute **FIGS. 7, 8, 9-10, 12, 19 and 23** attached at Exhibit Tab A of the accompanying Submission of Substitute Drawings.

IN THE SPECIFICATION:

Please amend the specification as follows:

Amend the paragraph at lines 9-11 on page 16 of the specification, as indicated in the accompanying Exhibit 1, so that the paragraph reads as follows:

FIG. 7 is an example of an *in vitro* method of overlap extension reassembly, targeting identified crossover locations. The appropriate fragments may be obtained by split-pool synthesis. In **FIG. 7**, part (A), all possible recombinants are prepared by crossover at positions 1 and 2. In **FIG 7**, part (B), the recombinants can be prepared by assembly of synthetic fragments containing the crossover positions. This example requires fragments (plus end primers).

Amend the paragraph at lines 12-15 on page 16 of the specification, as indicated in the accompanying Exhibit 1, so that the paragraph reads as follows:

FIG. 8, part (A), shows a fragment reassembly method using a parental template. The synthetic fragments are extended against a parent template strand and the gaps are repaired. In **FIG. 8**, part (B), the resulting products are subjected to heteroduplex recombination (Volkov *et al., Nucl. Acids Res.*, 27:18 (1999))to create libraries of genes within regions of non-identity. More complexity can be introduced by the addition of more fragments during template assembly.

Amend the paragraph at lines 16-17 on page 16 of the specification, as indicated in the accompanying Exhibit 1, so that the paragraph reads as follows:

FIG. 9 shows the preparation of gene fragments prepared by PCR with primers directed to regions targeted for crossovers. In **FIG. 9**, part (A), the fragments are prepared by PCR with primers. The PCR reactions are performed with primers 1 + 2, 3 + 4 and 5 + 6. The method is repeated for the other parents.

Amend the paragraph at lines 18-19 on page 16 of the specification, as indicated in the accompanying Exhibit 1, so that the paragraph reads as follows:

FIG. 10 shows recombination directed to specific sites using crossover primers in DNA shuffling. In **FIG. 10**, part (A), crossover primers designed to have crossovers at designated positions (2 primers for each position) are prepared. In **FIG. 10**, part (B), the parent genes are fragmented and reassembled, utilizing PCR methods, in the presence of the crossover primers to promote recombination at designated positions.

Amend the paragraph at lines 22-23 on page 16 of the specification, as indicated in the accompanying Exhibit 1, so that the paragraph reads as follows:

FIG. 12 is a flow diagram illustrating one embodiment of a recombinant search algorithm of the invention, based upon sequence identity. In **FIG. 12**, part (1), the parent sequences are aligned with the template structure. In **FIG 12**, part (2), all possible crossover points are determined according to a sequence identity algorithm . In **FIG. 12**, part (3), the coupling matrix is calculated. In **FIG. 12**, part (4), a start

parent is picked at random and copied to the offspring until a possible cut point is reached. In **FIG. 12**, part (5), a random number is picked, and if the number is less than *p*, a random new parent is copied until the next cut point is reached. In **FIG. 12**, part (6), the crossover disruption of the offspring gene is determined.

Amend the paragraph beginning at lines 22 on page 17 of the specification, as indicated in the accompanying Exhibit 1, so that the paragraph reads as follows:

FIG. 19 is a schematic demonstrating the utility of a contact map in identifying compact units of substructure. A representative contact map is on the left. The graph on the right is a statistical study of the average length of contiguous residues that can fold into a sphere of the indicated diameter (Gilbert 1998). This information can be used in the following way. If a 15-residue segment can fold into a sphere with a diameter of 21 angstroms, then this segment could be considered as being of average compactness. However, if a 20-residue segment can fold into a sphere of 21 angstroms, this is considered as having a significantly above-average compactness. This is visualized on the contact map as a triangle on the diagonal formed by the cut points required to generate the segment. If the segment fits into a sphere of the specified diameter, then the triangle will be entirely white (interacting). The contact map shows residues that are distant (black) and residues that are close

(white). If a given segment, [redacted], folds an above average number of residues into a given sphere size, then it is compact.

REMARKS

The specification and Figures for this application have been amended in order to comply with the requirements for drawings under 37 C.F.R. § 1.84(o). In particular, the drawings have been amended to remove excessive text. Accordingly, the specification has also been amended to reflect these changes to the drawings. In particular, the Brief Description of the drawings has been amended to incorporate the description originally provided as part of the drawings.

The above made amendments do not introduce new matter to the present application. Accordingly, entry of these amendments is respectfully requested.

Respectfully submitted,

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